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EFFECT OF ADENOSINETRIPHOSPHATE (ATP) ON THE ENDOGENOUS OXYGEN UPTAKE OF DEVELOPING GRASSHOPPER EMBRYOS[1]

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A phosphate transfer system has been found in practically every tissue thus far investigated (Lardy, 1949). It functions directly or indirectly in almost every phase of metabolism and has become so well established that its presence and functions are very often inferred without further demonstration. Previous studies in this laboratory have revealed that homogenates of the embryos of the grasshopper, *Melanoplus differentialis,* in 0.25 *M* sucrose can oxidize hexose phosphates to a greater extent than glucose (Bodine and West, 1953). Bodine and Thompson (1938) reported that labile phosphate is found in both the embryo and yolk while Lu and Bodine (1953) found a gross transformation of phosphorus from yolk to embryo. However, very few direct observations seem to have been made on the chemical or functional nature of the phosphate transfer system in developing organisms. Recently Albaum and Kletzkin (1948) and Calaby (1951) confirmed the presence of ATP in insects. Humphrey and Siggins (1949) presented indirect evidence that glycolysis in insect muscle involves the phosphate transfer system while Sacktor (1953) describes a specific ATPase in flight muscle mitochondria.

The present paper is concerned with results of a study on the effects of ATP on the endogenous O_2 uptake of grasshopper embryos at different developmental stages. These results are discussed in the light of a functional phosphate transfer system as exhibited by other organisms.

Materials and Methods

Embryos of the grasshopper, *Melanoplus differentialis,* were dissected from eggs in Ringer solution (buffered at pH 6.8 with *M*/15 phosphate) and washed free of adhering yolk (Bodine and Boell, 1934, 1936). The washed embryos, suspended in a suitable volume of the selected medium, were homogenized using a Pyrex glass tube with a tight fitting selenite rod as a pestle. The pestle rotated at 1150 r.p.m. and the time of homogenation was two and one-half minutes at 0° C.

Two suspension media were used, Ringer solution containing 0.0035 *M* mag-

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nesium chloride (pH 6.8) and 0.25 *M* sucrose containing 0.0035 *M* magnesium and 0.0035 *M* calcium chlorides and 0.03 *M* phosphate (pH 6.8) (Bodine and West, 1953). One hundred intact embryos or homogenates containing the equivalent of one hundred embryos per cubic centimeter were used throughout this investigation.

Oxygen uptake determinations (air as gas phase) were carried out by standard Warburg techniques at 25° C.; 0.5 cc. of substrate were tipped from the sidearm to make the final volume of the reactants 1.5 cc.

Adenosinetriphosphate (ATP) (sodium salt) was obtained from the Sigma Chemical Company, St. Louis, Missouri.

Results

The effect of ATP on the endogenous O_2 uptake of intact embryos (mitotically active or blocked) was investigated over a range of concentrations from 2.5 to 10.0 μmoles per 1.5 cc. Data for a typical experiment are summarized in Table I. From an examination of this table it is apparent that ATP has little, if any, sig-

Table I

	Prediapause (17 days)		Diapause (40 days)		Postdiapause (3 days)	
	E	H	E	H	E	H
Control (sucrose)	17.0	5.7	10.0	4.6	17.4	5.9
ATP	16.0	9.4	9.3	6.5	16.5	13.3
ATP+glu	15.0	10.2	9.1	8.0	16.7	14.3
ATP+glu−1−PO_4	19.6	13.2	14.2	9.7	18.2	13.6
Glucose−1−PO_4	18.8	8.5	15.2	6.5	18.5	9.0

Shows O_2 uptake (cc.) for 100 minutes for prediapause, diapause and postdiapause embryos (E) and their homogenates (H) in 0.25 *M* sucrose plus Mg^{++} and Ca^{++} after addition of ATP, 5 μmoles per flask; glucose 1.0%; glucose-1-phosphate 0.5%. Stimulation due to hexosephosphate has previously been pointed out (Bodine and West, 1953). Data in table are taken from one series of experiments and represent averages from a minimum of 8 determinations. All data from different experiments have been statistically analyzed and differences, indicated in text, found to be significant.

nificant stimulating effect on the respiration of intact embryos either in 0.25 *M* sucrose or Ringer solution. This lack of effect may be related to or conditioned by the permeability of the intact embryo to these reagents.

The effect of ATP on the endogenous O_2 uptake of homogenates of embryos in 0.25 *M* sucrose is strikingly different from that of the intact embryo. ATP augmented the endogenous respiration of homogenates in sucrose (Table I). The concentration effect was found to be quite variable at high concentrations and this is attributed to the formation of clumps which entangled the mitochondrial elements, thus preventing or interfering with electron transfers. This clumping effect was more apparent in diapause and postdiapause stages at the 10 μmole level of ATP. Clumping is believed to be caused by an involvement of embryonic actin, myosin, and ATP and is given support by the observation that clumping seldom occurred in the prediapause stages before 17 days at which time the percentage

stimulations were more consistent. Maximal augmentation of respiration was obtained at the 5 μmole level where the clumping effects were absent. This concentration has been selected as optimal in these experiments. ATP, when tipped from the sidearm, produces a lag before maximum augmentation of oxygen uptake. Homogenates made in 0.25 *M* sucrose plus ATP (employed only for diapause) showed a greater oxygen uptake than when ATP was added to the sucrose homogenate. The magnesium ion was necessary for maximal stimulation by ATP.

Homogenates made in Ringer and Mg^{++} showed no stimulation of endogenous oxygen uptake when ATP was added.

Combinations of ATP and glucose produced no marked hexokinase activity in either the intact embryo or its homogenate. Similarly, no marked phosphoglucokinase activity was apparent.

Washed nuclei in sucrose or Ringer showed no response in their endogenous oxygen uptake to these concentrations of ATP.

Discussion

The exact nature of the labile phosphorus compounds of the phosphate transfer system in this material has not yet been satisfactorily demonstrated, due largely to various inherent technical difficulties. However, it is known that the labile phosphorus component of the embryo is adsorbed on activated charcoal (method of Crane and Lipmann, 1953), which is a characteristic of the adenosine-containing nucleotides (unpublished data). The ability of hexosephosphates to stimulate endogenous respiration of intact embryos is quite unusual and no active mechanism has been revealed (Bodine and West, 1953). ATP, unlike the hexosephosphates, seems to have no stimulating effect on the endogenous respiration of the intact embryo (mitotically active or blocked). Similarly, glucose plus ATP gave no increased endogenous O_2 uptake, indicating no marked hexokinase activity at or near the cell membrane.

ATP markedly stimulates endogenous respiration of the homogenates in 0.25 *M* sucrose (Mg^{++}, Ca^{++}) and thus one can infer a functional phosphate transfer mechanism. This effect may take place through "active" phosphorylation of endogenous substrates, making them more available for oxidation, or "active" dephosphorylation by a specific ATPase, increasing the concentration of high energy phosphate acceptors (ADP + AMP) and permitting the oxidation of available endogenous substrates or a combination of both. (This discussion presupposes that oxidation and phosphorylation are linked.) Studies are in progress to clarify this point.

ATP does not stimulate the endogenous respiration of Ringer homogenates. The mitochondria lose their morphological integrity in this medium and show a marked functional difference to added succinate and hexosephosphates. Thus structural integrity of the mitochondria in this material seems related to their functions.

Combinations of glucose or hexosephosphates with ATP in sucrose homogenates yield variable results. No effort was made to remove the endogenous substrate, and at present it can be said that there appears to be no marked hexokinase or phosphohexokinase activity in this material.

Summary

1. A study has been made on the effects of ATP on the endogenous O_2 uptake of grasshopper embryos and homogenates at different developmental stages.
2. ATP has little, if any, effect upon the O_2 uptake of the intact embryo.
3. ATP augments the O_2 uptake of homogenates in sucrose.
4. The magnesium ion is necessary for maximal stimulation of ATP.

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